

Summary Report: What Clinical Laboratories Need to Know About Their Role in Influenza Testing*

Laboratory Outreach Communication System
Inaugural Teleconference
Tuesday, January 24, 2006

Introductory Remarks

*Robert Martin, DrPH, CDC
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Today's teleconference is the initial conference call of the Laboratory Outreach Communication System (LOCS). The system is modeled after CDC's Clinical Outreach Communication Activity (COCA), which provides a national forum for CDC to communicate regularly with clinicians on a range of important current issues. Objectives of today's meeting are to introduce LOCS, describe the system in place to address influenza testing (clinical/state public health laboratory/CDC), and to articulate the role and responsibilities of the clinical laboratory.

LOCS is being established to address existing gaps in laboratory-related communication with the broad clinical community. The vision for the system is to be the central source for laboratory professionals to obtain credible information on routine and emergent issues. LOCS project statement is "to build a volunteer communications infrastructure for the exchange of laboratory-related information between CDC and others in the laboratory community.

LOCS activities will focus on education, using technology that enables two-way communication between CDC and participating partners. One of the key objectives is to provide up-to-date information on dynamic or urgent public health issues – as seen with today's program on laboratory diagnostics for influenza. Key components of LOCS communications efforts include the following:

- Audience – Public health, clinical, physician office, independent laboratory staff
- Timing – Scheduled and unscheduled
- Route of communication – Teleconference, web conference, mailing lists, email, phone/FAX
- Topics – Needs of laboratorians that are not being met, emergent issues, changes in regulations, standards, recommended practices, disaster relief.

Today's program features speakers from CDC's Influenza Branch and, on behalf of APHL, from the Wisconsin State Laboratory of Hygiene.

Routine and Enhanced Surveillance for Influenza at the National Level

Lynnette Brammer, MPH, CDC

Surveillance is a cornerstone of CDC's public health programs, and over the years the Influenza

This report reflects author's notes and recollections of the meeting, and the views expressed herein may not necessarily represent the positions and policies of the Centers for Disease Control and Prevention (CDC) or the Department of Health and Human Services (HHS).

Branch has established a comprehensive system to monitor the occurrence of seasonal influenza in the United States. The system consists of the following seven components:

- **World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS) Collaborating Laboratories:** About 75 WHO and 50 NREVSS collaborating laboratories located throughout the United States report the total number of respiratory specimens tested and the number positive for influenza types A and B each week. Some of the influenza viruses collected by laboratories are sent to CDC for more testing.
- **U.S. Influenza Sentinel Providers Surveillance Network:** Each week, approximately 1,000 health-care providers around the country report the total number of patients seen and the number of those patients with influenza-like illness (ILI) by age group. For this system, ILI is defined as fever (temperature of $>100^{\circ}\text{F}$) plus either a cough or a sore throat.
- **122 Cities Mortality Reporting System:** Each week, the vital statistics offices of 122 cities report the total number of death certificates filed and the number of those for which pneumonia or influenza was listed as the underlying or as a contributing cause of death. The percentage of all deaths due to pneumonia and influenza are compared with a baseline and epidemic threshold value calculated for each week.
- **State and Territorial Epidemiologists Reports:** State health departments report the estimated level of influenza activity in their states each week. States report influenza activity as no activity, sporadic, local, regional, or widespread.
- **Influenza-associated pediatric mortality:** Influenza-associated pediatric mortality is a newly added nationally notifiable condition. Laboratory-confirmed influenza-associated deaths in children <18 years old are reported through the Nationally Notifiable Disease Surveillance System.
- **Emerging Infections Program (EIP):** The EIP Influenza Project conducts surveillance for laboratory-confirmed influenza related hospitalizations in persons <18 years in 57 counties covering 11 metropolitan areas of 10 states. Cases are identified by reviewing hospital, laboratory, and admission databases and infection control logs for children with a documented positive influenza test (culture, DFA/IFA, PCR, or a rapid test) conducted as a part of routine patient care.
- **New Vaccine Surveillance Network (NVSN):** The New Vaccine Surveillance Network (NVSN) provides population-based estimates of laboratory-confirmed influenza hospitalization rates for children <5 years old residing in 3 counties (Hamilton County OH, Davidson County TN, and Monroe County NY). Children admitted to NVSN hospitals with fever or respiratory symptoms are prospectively enrolled and respiratory samples are collected and tested by viral culture and RT-PCR. NVSN estimated rates are reported every 2 weeks.

Together, the seven influenza surveillance components are designed to provide a national picture of influenza activity. Pneumonia and influenza mortality is reported on a national level only. Sentinel physician and laboratory data are reported on a national level and by influenza surveillance region. The state and territorial epidemiologists' reports of influenza activity are the only state-level information reported. Both the EIP and NVSN data provide population-based,

laboratory-confirmed estimates of influenza-related pediatric hospitalizations but are reported from limited geographic areas.

In February 2004, following reports of human infections with avian influenza A (H5N1) virus in Asia, CDC issued a national advisory recommending enhanced surveillance and laboratory diagnostic criteria. The interim guidance is intended to target potential at-risk persons, including travelers entering the United States from H5N1-affected countries (e.g., Vietnam, Thailand, Cambodia, China). Patients with fever (temperature of 38°C [100.4°F] or greater), travel history to an H5N1-affected country, and history of direct contact with diseased, dead, or infected birds within 10 days of symptom onset can be considered for testing on a case-by-case basis, in consultation with public health officials. Influenza A (H5N1) is a select agent, and special laboratory precautions should be observed. Procedures involving isolation and live-virus work must be done under conditions of biosafety level 3 (BSL-3) with enhancements. RT-PCR assays can be done in BSL-2 facilities. Training in RT-PCR testing for state laboratory personnel has been conducted at CDC, in conjunction with APHL. State laboratories that cannot conduct H5N1 testing can send specimens to CDC for analysis. Results will usually be available within 24 hours.

Rapid Diagnostic Tests for Influenza:

What the Clinical Laboratory Should Know

Peter A. Shult, PhD, Wisconsin State Laboratory of Hygiene

Clinical laboratories are now assuming a major role in the laboratory diagnosis of influenza. For a subset of these laboratories this role is carried out using the more traditional methods of influenza diagnosis: virus culture, antigen detection by immunofluorescence, and PCR.

More and more laboratories, however, are employing one of an ever-expanding variety of rapid antigen detection (basically EIA or EIA-like tests) methods that enable the rapid laboratory diagnosis of influenza, often within a half hour. There are now 13 kits capable of detecting:

- influenza A virus only,
- both influenza A or B without identifying the type,
- both influenza A or B and identifying the type

Currently, none of the FDA-cleared tests can differentiate influenza A subtypes. Moreover, since a number of these kits have “waived” CLIA status, they can be performed in non-laboratory (non-traditional) settings. In fact, it is my experience (at least in Wisconsin) that the vast majority of these kits are being employed in these settings (POs, nursing homes, emergency rooms, Urgent care offices).

The potential impact of these tests in clinical practice is obvious. Less obvious perhaps is the impact these diagnostic tests may have on public health response both to seasonal influenza (i.e., our annual influenza epidemic) and to emergence of novel strains which have the potential to cause the next pandemic.

In this context then, the objectives here are to:

- Review briefly the advantages of and concerns with these rapid diagnostic methods,
- Suggest how the concerns might be addressed to optimize test performance and results interpretation and, most importantly,

- Suggest throughout the talk how the PHL can be a resource for helping optimize the use of these tests and provide critical linkage between the testing sites and CDC's influenza surveillance and response efforts (for both "seasonal" and "novel", potentially pandemic strains of influenza)..

Suggested **advantages** of these rapid diagnostic tests are clear and obvious:

- Easy to perform; little expertise required
 - Provide widespread testing outside of typical laboratory realm
- Rapid turn around time
 - To aid diagnosis and patient management
 - guide patient treatment decision (antivirals/antibiotics) in a clinically relevant timeframe
 - enable infection control measures to prevent transmission in health care and other institutional or particular community setting
- Relatively inexpensive

However, I want to really focus on some of the important **concerns** in the use of these tests and how these concerns might be addressed:

Performance limitations

- Problem: Low sensitivities reported with some kits
 - Data from kit inserts and *Uyeki TM. Pediatr Infect Dis J 2003; 22: 164-77*
 - Not a lot of comparative data; difficult to compare across studies or kit inserts
 - Median sensitivity of 70-75% when compared with cell culture or IF. Anticipate lower when comparing to RT-PCR
 - Reasons related to:
 - inherent test limitations
 - specimen type (especially use of throat swabs; nasopharyngeal specimens especially aspirates/washes are optimal)
 - specimen quality (when specimen taken, how taken, how handled as with any test)
 - age of patient (better performance in children who shed more virus for longer period)
 - Performance concerns with detection of novel strains (e.g., H5N1) where preliminary reports of poor sensitivity compared with PCR and culture. Related to different virus shedding patterns? This not for certain yet.
 - On the other hand, **specificities** have been reported to be quite good, in general **90-95%**
 - Solutions: Make better kits, choose best kit, control specimen quality (type, timing and technique)
- Problem: Predictive Values (Note: Refer to discussion of this in Appendix 6 of Supplement 2 [Laboratory Diagnostics] of the *HHS Pandemic Plan*)
 - **Predictive value** is the probability of the presence or absence of disease given the results of the test
 - Dependent on test performance characteristics(sensitivity and specificity) and prevalence (current level of activity in the community. Going through simple mathematical analyses will demonstrate this.
 - Predictive Value Positive - dependent upon test specificity

- Despite good specificity, **very poor PVP** (false positives) during periods of low prevalence (i.e., early or off-season testing) in a typical flu season (unfortunately when many want to use these tests).
- Could cause considerable problems for public health response if used during period of likely very low prevalence just following emergence of a novel subtype when concerns with pandemic potential would be heightened. Last thing we'd need is false positive results!
- PVP, however, will improve considerably when these kits are used during periods of high flu prevalence (near the epidemic peak)
- Predictive Value Negative – dependent upon test sensitivity
 - With the relatively poor sensitivity (discussed above), the **PVN** (false negatives) will pose a problem the closer we get to peak influenza activity. This of particular concern given the likely reliance on these test results for treatment of patients with antivirals, both during seasonal influenza or if a novel subtype emerges and demonstrates sustained human-to-human transmission in the human population
 - PVN, however, is very good, early in the flu season or off-season
 - During this period we should recognize the value of negative results (e.g., in an institutional setting experiencing out break of severe respiratory disease)
- Rapid test results need to be interpreted in the context of the patients clinical presentation and the current influenza epidemiological picture.
- Solutions: Use of these tests can be optimized by...
 - Educate clinicians on PVs and limitations of test results
 - Confirm early, late and out-of-season positives
 - Confirm peak-season negatives if warranted
 - Use “prevalence indicators” to decide:
 - When to test
 - When to qualify result
 - When to confirm result

NOTE: Role for the PHL: training, confirmatory testing, surveillance data

Biosafety concerns

- Problem: A second area of concern is Biosafety. Why is this?
 - Continuous emergence of new threats (SARS, avian flu, H2N2)
 - Use of tests by non-laboratorians without sufficient knowledge of or regard to BSL
 - Even for laboratorians it is convenient to perform test outside of BSC or outside of normal BSL-2 protocols; lack of BSCs
 - Potential aerosol generating steps
 - Solution: Clinical labs and POC testing sites need to re-think biosafety in age of EIDs for all diagnostic testing.
 - Need for risk assessment prior to specimen collection and testing and convey this information to the laboratory
 - Travel Hx, exposure Hx, pathogenicity, route of Tx, infectious dose, etc (Get slide)
 - Enhance communication with ID Drs., Infection Control
 - Strategies to enhance Biosafety in lab, testing sites
 - Use of BSCs, PPE and or barriers, sequestering work,

- For labs, power of “strict” BSL-2 practices
 - Specimen referral to higher biocontainment
 - Need for basic training particularly for non-laboratorians

Testing kit supplies

- Problem: Reports of supply disruptions during recent influenza seasons. Near certainty this would occur with emergence and sustained transmission of novel influenza subtype
- Solution: No easy solution. Perhaps maintain adequate stockpile and perhaps consider evaluation/validation of different kits

Loss of surveillance data and viral isolates needed for further characterization

- Problem: With widespread rapid testing and diversion of testing from PHL, potential loss of testing data (in many states influenza is not reportable) and, more importantly, potential loss of follow-up testing and characterization of virus isolates necessary for:
 - for monitoring influenza A subtypes and strains circulating in the U.S
 - detecting novel influenza A subtypes
 - for strain analysis needed for annual vaccine selection
 - monitoring potential emergence of resistance to antiviral drugs
- Solution: State PHL should lead development of laboratory and other testing site networks and these testing sites should seriously consider participation for the purposes of:
 - Collection of influenza diagnostic testing data
 - Number of specimens tested/week; number and ID of positives
 - Acquisition and further testing (by culture or PCR) of patient specimens for :
 - confirming rapid test results
 - obtaining influenza isolates for further characterization (see above) at PHL or CDC
 - Identifying which other pathogens may be involved
 - PHLs may be in a unique position to carry out these activities due to:
 - Historical participation of many (most) state PHLs as W.H.O. Collaborating Laboratories for influenza surveillance (reference testing, seasonal influenza and novel strain surveillance, antiviral susceptibility)
 - State PHL are designated lead LRN “Reference” labs within each state and funded to:
 - Build a Sentinel Lab network for response to BT and other public health threats
 - Develop or expand state-of-the-art diagnostic capabilities and capacities
 - Develop and maintain courier system
 - Provide laboratory-based training opportunities
 - Emergency communication systems
 - PHL historical role as close collaborator with and liaison to CDC in diagnostic testing and outbreak response matters

The PHL can and should be the clinical lab’s most appropriate link to CDC and best immediate source for consultation during outbreaks of influenza or other public health emergency.

Selected references:

- HHS Pandemic Influenza Response Plan: www.pandemicflu.gov
- FDA article entitled “Cautions in Using Rapid Tests for Detecting Influenza A Viruses: www.fda.gov/cdrh/oivd/tips/rapidflu.html
- Uyeki TM: *Pediatr Infect Dis J* 2003;22:164-77
- www.aphl.org/programs/infectious_diseases/pandemic_influenza.cfm

What Are State and CDC Roles in Public Health Response?

Niranjan Bhat, MD, Influenza Branch, CDC

The purpose of this talk is to reinforce and build upon points made by previous two speakers.

- Case definition
 - Case definition means required features that should trigger H5-specific testing of a patient
 - Also leads to initiation of biosafety measures in lab, public health investigation, clinical management including infection control and presumptive treatment
 - Must be specific enough to limit number of evaluations
 - e.g., flood of cases after first H5 cases identified in US, or a cluster of suspected cases
 - Clinical labs will be flooded with specimens from patients with human influenza, other resp viral diseases that may be concurrently circulating, substantial numbers of worried well
 - Even now, when numbers are small, important to save resources
 - Clinical labs must use case definition to prioritize specimens, initiate biosafety procedures, encourage/educate providers to use
 - Case definition must emphasize the following
 - returned traveler from an H5N1-affected country
 - an influenza-like illness (documented fever + respiratory symptoms)
 - a severe illness (hospitalization, pneumonia)
 - an exposure (contact with poultry or suspect human case)
 - Case definition is not rigid; for example, would consider testing if:
 - severely ill, but can't get an exposure history
 - mild illness, but strong exposure history
 - Components of case definition are subject to change as we learn more
 - spectrum of disease may include mild forms, or non-respiratory illness
 - new animals may be risk factors
 - may become unassociated with animal outbreaks
 - must keep up to date with the CDC's website
- Role of public health laboratories
 - From our perspective, important to involve public health agencies asap to:
 - advise on testing and management
 - initiate public health investigations and control of potential spread
 - interact with the media and public concern
 - We would therefore encourage providers to send specimens directly to state public health lab, but recognize:
 - providers may prefer their affiliated clinical lab

- suspicion of H5N1 may develop after specimens are sent
- From a provider's point of view, return of results is comparable
 - if state PH lab is only a few hours' drive away
 - otherwise, overnight delivery to state or CDC lab
 - commercial labs often ship overnight to a single location
 - upon arrival, RT-PCR testing may only take a few hours
 - inconclusive/discordant results may lead to re-testing and delays, but this could and should happen in both commercial and public health labs
- State health departments are in the best position to manage suspect H5N1 investigations
 - majority of state labs have RT-PCR capacity and training
 - if unable or trouble interpreting, can forward to CDC overnight
 - state epidemiologists are able to mobilize local resources
- State health departments and CDC provide technical assistance on:
 - lab technique and interpretation of results
 - verification and confirmation of results
 - epidemiologic/public health investigations
 - advice on clinical management, infection control
 - communication with clinicians, laboratories, public health community, media, policy-makers, domestic and international public
 - serve as central clearinghouse for information on: current geography, clinical spectrum, and transmissibility of H5N1
 - communication is a 2-way interaction - PH can learn from the clinical labs
- Pitfalls and points to consider
 - please communicate potential positive results to both the state public health lab and the state communicable diseases epidemiologists - otherwise, more complicated chain of communication
 - please contact public health before media is alerted (provider and patient must be cautioned about this)
 - H5N1 is a moving target
 - CDC keeps up with evolving strains
 - CDC supports sharing of diagnostic testing information with clinical laboratories, but capacity is limited for providing individual technical assistance
 - testing for H5N1 is a big responsibility for a laboratory
 - biosafety procedures
 - quality control
 - post-exposure monitoring and management of employees

Questions and Answers

Note: Responses to questions raised during and after the LOCS conference call are intended to provide immediate information about what is known at the time. However, the answers have not been reviewed and cleared and do not necessarily reflect the official views of CDC and HHS.

Q. Can you provide more information about COCA?

A. CDC's Clinician Outreach Communication Activity (COCA) has established partnerships with national clinician organizations for the purpose of timely communication of information on disease outbreaks and terrorism events. Conference calls are conducted on a regular basis. For more information, see the COCA website at <http://www.bt.cdc.gov/coca/>

Q. Regarding rapid diagnostic tests: why they are less sensitive to influenza H5 than to influenza A H1 or H3?

A. The primary reason appears to be lower viral load.

Q. Why are consensus sequence data available only to public health laboratories?

A. CDC receives human samples under a variety of stipulations and restrictions, including requirements that information first be released to the submitting country. Countries submitting material to CDC for testing frequently do not agree to release of sequence data.

Q. Clinical laboratory staff have an important role in public health response, as indicated during today's presentations. Will CDC recommend that laboratory workers be included in priority groups for vaccination?

A. The development of recommendations for vaccine priority groups in an open, ongoing, and collaborative process. CDC program personnel consult with other subject matter experts in considering a wide range of factors related to establishing priority groups. After thorough deliberations, decisions are made by the Advisory Committee on Immunization Practices.

Q. Which state public health labs are currently receiving samples and able to type them for H5N1?

A. According to APHL estimates, approximately 60 state and local (county and large city) laboratories have the ability to test for H5.

Q. If independent academic or reference laboratories receive a positive test result for H5N1, should that result (and sample) go to the state lab (for consultation and confirmation) before reporting to a physician?

A. Yes, under these circumstances the result and sample should be sent to the state lab or CDC for confirmation before reporting to a physician or other care giver.

Q. How discordant have different molecular identification methods been for H5N1?

A. Except for technical differences, different methods are using different sequence databases, which may be incomplete or not up-to-date. This means that some test protocols may miss some groups of viruses.

Q. Why can't the CDC primer set be shared (not the sequence data from partner countries that may have been foundational for their design)?

A. There are legal issues related to this. CDC has been working with FDA to obtain clearance for the CDC real-time RT-PCR assay for identification of influenza A H5 (Asian lineage). When approved, the reagents will be available for LRN-designated laboratories nationwide. (Note: FDA cleared the assay on February 3, 2006. See <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm55e203a1.htm>)

Q. Do H5N1 molecular typing requests to state labs require a signed consent (similar to SARS)?

A. After the test has been cleared by FDA, the consent form will not be needed.

Q. Other than web sites, who can we contact for specific information on H5N1 laboratory diagnostics?

A. H5N1 laboratory testing is a rapidly developing and changing topic. Technical information can be obtained in articles published in scientific journals.

Q. What test material is available for H5N1 molecular test validation? Will updated test materials become available to accommodate the need to detect evolving H5N1 strains?

A. See below for information regarding the availability of the inactivated influenza A/H5 virus from which H5 positive control RNA can be isolated and used in PCR or real-time PCR. The evolution of H5 viruses may require modification of the real-time PCR protocol and, if necessary, of the positive control sample. CDC performs enhanced surveillance and follows up the evolution of H5 viruses and will make necessary modifications when needed.

- The inactivated influenza A/H5 control material for RT-PCR testing of avian influenza (A) H5N1 (Asian lineage) is currently available to U.S. public health laboratories at no charge and is available to international government laboratories at no charge. The influenza A/H5 control material also is available to all commercial scientific and manufacturing entities in the United States and internationally.
- Due to limited supply and high demand, laboratories initially will be restricted to one vial. Each vial contains 500 ul of control material that should provide enough RNA for approximately 1,000 reactions. Requests for additional material will be considered on a case-by-case basis. To obtain the control material, please follow the procedure outlined below.
 - When placing an order, provide the catalog number (i.e., VA2711 05-0180) and description of the product [i.e., influenza A (H5N1) (Asian lineage) real-time RT-PCR control]. The number of vials is limited to one per order.
 - The order should be written on the facility's letterhead.
 - Provide a complete mailing address and phone number for shipping. The requesting official should provide his name and title.
 - The order must be sent to CDC (ATTN: Avian flu control material, "NCID/SRP" via fax (404-639-3086)). E-MAIL ORDERS WILL NOT BE ACCEPTED.
 - Allow 10 business days for delivery. Shipments are sent out to arrive during the same business week; therefore, orders placed late in the week or before a holiday will not be shipped until the following week.